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REVIEW

# Prognostic value of soluble major histocompatibility complex class I polypeptide-related sequence A in non-small-cell lung cancer – significance and development

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**Abstract:** Soluble major histocompatibility complex class I polypeptide-related sequence A (sMICA) is a useful marker in surveillance of lung cancer. High serum sMICA level in patients with non-small-cell lung cancer (NSCLC) seems to be a poor prognostic factor being correlated with poor differentiation and advanced stage. However, the low specificity limits its role as a single prognostic marker of NSCLC, but its evaluation, in addition to standard serum markers, could improve the staging of NSCLC. Despite promising, all current studies are insufficient to assess the real efficiency of sMICA as a prognostic marker of NSCLC, and hence, future studies are required to validate it.

**Keywords:** NSCLC, MICA, MICB, prognostic factor

## Introduction

The human major histocompatibility complex (MHC) comprises a cluster of genes mapping to the short arm of chromosome 6. In 1994, a new family of polymorphic genes that map within the MHC class I region was described.[1] This family, named "MHC class I chain-related", includes two functional genes: MHC class I polypeptide-related sequence A (MICA) and MHC class I polypeptide-related sequence B (MICB).[2] Typically, MICA encodes for a polypeptide of 383 amino acids that is expressed on the cell surface of different cells. In patients with advanced-stage non-small-cell lung cancer (NSCLC), elevated expression of MICA on the cell is associated with poor prognosis. After DNA damage, the cell reacts with an overexpression of MICA. NK group 2-member D (NKG2D) is a receptor of MICA and is localized on the cell surface.[3] The MICA–NKG2D pair is a versatile system since NKG2D can act as a primary receptor or co-stimulatory molecule during antitumor immune responses by all natural killer (NK) cells. However, the tumor cells protect themselves through a particular mechanism: a soluble form of MICA (sMICA) is released and, thanks to the proteolytic action of metalloprotease, sheds from the tumor cell surface. The final step is the induction of downregulation of NKG2D on cell surface.[4–6] In fact, when NKG2D is bound together with sMICA, the expression of NKG2D is downregulated; this mechanism allows the tumor to escape from the host immune system since it produces the outgoing NK/T cell activation trigger.[7,8] If sMICA could be a diagnostic and prognostic factor in patients with NSCLC remains unclear. Thus, the aim of the present review is to evaluate the prognostic role of sMICA in NSCLC, and its significance and development.

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## Pathophysiology of sMICA serum levels in NSCLC

The molecular structure of MICA is not associated with β2-microglobulin nor with the peptide-binding groove that is too tight to suit a ligand. Therefore, MICA cannot be considered as an antigen-presenting molecule, but it should be treated as a cell homeostasis sensor, and not a cell stress sensor. Its upregulation is induced by a strong cell proliferation and pro-inflammatory stimulus that will disrupt the cellular homeostasis and elicit a cytotoxicity that eliminates altered cells, contributing to the restoration of the normal homeostasis.[9] NKG2D, the MICA receptor, may act as a co-stimulatory molecule or a primary receptor involved in target cell recognition, above all NK cells. Despite this evidence in vitro, in vivo, the correlation between tumor aggressiveness and the role of MICA in control of tumor growth remains underestimated. All human NK cells, δγ T lymphocytes, and αβ CD8+ T lymphocytes express NKG2D on their surface as a homodimer; the analysis of the crystal structure of the MICA–NKG2D complex revealed that NKG2D binds as a homodimer to one molecule of MICA.[10] It has been demonstrated that the NKG2D–sMICA tie induces a downregulation of NKG2D expression on the surface of cells, but this is a reversible process that imposes a functional impairment to the immune surveillance exerted by NK cells, γδ T lymphocytes, and αβ CD8+ T lymphocytes.[7] In many instances, there is a safeguard mechanism which protects the T cells from NK cytotoxicity during a T cell-dependent immune response in tumor microenvironment: this mechanism consists in MICA retained inside the T cell. In fact, during an inflammatory process, NK and activated T cells are recruited and further stimulated with locally produced cytokines. Although activated T lymphocytes can be killed by NK cells,[11] an extra signal may be necessary to express MICA on the activated T cells like other cell populations presented in inflamed, virus-infected, or neo-transformed tissues. Normal human tissues do not express MICA or its expression is rare, while MICA expression has been found in different epithelial and non-epithelial tumor cell lines including the lung, breast, kidney, ovary, prostate, and colon carcinomas, melanomas, and acute myeloid leukemia.[8,12–20] MICA/B overexpression appears to be related to the activation of the DNA damage pathway, and it has been proposed to play a role in human tumor rejection.[21] The presence of NKG2DL on cells surface marks them for elimination by those immune effector cells which are all a type of NK cells, mostly NKT cells, γδ CD8+ T cells and γβ CD8+ T cells, as well as some γβ CD4+ T cells. These cells express the cognate NKG2D receptor for the NKG2D ligand (NKG2DL). Tumors secrete an sMICA that binds to NKG2D and downregulates its expression on the cell surface, leading to the loss of the NK/T cell activation trigger.[7,8] In this way, tumor could escape from the host immune system. Yet, sMICA/B has been reported to cause systemic downregulation of NKG2D. It is proposed that proteolytic shedding is the key mechanism by which MICA is released from the cell surface, and it is mediated by the activity of metalloproteases.[22–25] There are micro-RNA clusters that regulate surface expression and shedding. Metalloproteinases play an important role in invasion, metastasis, and angiogenesis processes of tumors, but they also have a role in allowing MICA shedding from tumor cells to evade immune attack. However, other different proteases can participate in the cleavage of MICA. Kaiser et al[26] demonstrated that MICA shedding was facilitated by a disulfide isomerase interacting directly with MICA α3. The levels and the polymorphic variants of this soluble molecule are correlated with tumor survival, staging, and grading. Thus, a qualification and quantification of these markers could be useful as diagnostic and prognostic factors.

## Overview of prognostic markers for NSCLC

Suzuki et al[27] investigated the association between immune response and clinical outcome in lung cancer, remarking the prognostic significance of immune markers in the tumor microenvironment as well as peripheral blood of NSCLC patients. The presence of tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment highly influences the behavior of immune response in cancer. Ruffini et al[28] showed that TILs, mostly CD8+, are correlated with prolonged survival but only in the squamous cell carcinomas. These results were in contrast with those of Wakabayashi et al[29] who found that CD8+ levels were associated with a shorter overall survival (OS), especially in adenocarcinoma. The role of stromal CD4+ and CD20+ cells, and co-localization of stromal CD8+ and CD4+ cells in improving OS in NSCLC is instead clear.[30] Lung cancer is the most frequently diagnosed cancer and the major cause of tumor-related mortality in the world. Generally, lung cancer is discovered when the disease is already at an advanced stage, and so the prognosis of these patients is very poor.[31–35] Thus, there is a need to establish the utility of known biomarkers that can facilitate a better

assessment of the response to specific therapies, improving the prognosis of patients with NSCLC.

Serum levels of marker proteins, including carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), carbohydrate antigen 19-9 (CA19-9), cytokeratin fragment 21-1 (CYFRA 21-1), CA15-3, CA242, and CA50, are commonly used to determine the prognosis of NSCLC.

## Carcinoembryonic antigen

In 1965, Gold et al identified CEA extracting it from human colon cancer tissue.[36] This molecule is a180-kDa transmembrane glycoprotein classified among the members of the immunoglobulin superfamily, expressed on cell surface, and involved in cell adhesion mechanisms. Despite the fact that CEA is ubiquitously expressed, higher levels in malignant tumor cells, including lung cancer, have been found. All histological types of lung cancer may produce CEA, but it has a more precise diagnostic value in adenocarcinoma than in small, large, or squamous cell carcinomas. A review by Grunnet and Sorensen[37] investigated the utility of CEA as a prognostic and predictive marker in patients with lung cancer: in 23 studies, the use of CEA serum level as a prognostic marker was evaluated in NSCLC, and in two studies, the use of CEA plasma level was evaluated. In 18 studies (17 serum, one plasma), CEA was found to be a useful prognostic marker for OS, recurrence after surgery, or/and progression-free survival in NSCLC patients. The serum level of tumor markers is important not only for their diagnostic values for screening of cancer patients but also as prognostic determinants for the selection of the optimal treatment modality for individual lung cancer patients, including the use of adjuvant chemotherapy.

## Neuron-specific enolase

It is the γ-subunit of enolase and has a major role in diagnosis and prognosis of small-cell lung cancer; however, its role as a marker in the NSCLC is not clear.[47,48] A study by Ferrigno and Buccheri[38] evaluated the relationship between the NSE serum levels measured before treatment and other clinical properties, such as CEA level. In this study, 448 patients with a diagnosis of NSCLC were enrolled, and NSE level was measured: in 32% of these patients, increased values of NSE were observed. Therefore, NSE is useful as a marker even in NSCLC and is a significant predictor of survival.

## Carbohydrate antigen 19-9

This molecule was isolated in 1979 by Koprowski from cells of human colorectal cancer. High level of CA 19-9 was found in many types of malignant tumors since it is not a tumor-specific but a tumor-associated antigen. Abdallah et al[39] investigated the role of CA19-9 in patients with advanced NSCLC. They evaluated the mean and median pretreatment level of plasma CA19-9 in 35 patients; the level of plasma CA19-9 was also measured in 34 healthy patients as controls. The results showed that there was a statistically significant difference between the patient and control groups ($p < 0.001$), demonstrating that CA19-9 can be a reliable marker for advanced NSCLC.

## Cytokeratin fragment 21-1

A fragment of cytokeratin 19, the CYFRA 21-1, was described as a useful marker in lung cancer patients in 1993. This soluble cytokeratin 19 fragment is mainly expressed in epithelial cells of malignant lung tumors, and its level could be determined using a sandwich enzyme-linked immunosorbent assay (ELISA). Studies by Lai et al[40] and Kao et al[41] showed that serum CYFRA 21-1 level was not correlated with sex and smoking habits, demonstrating that it is an independent prognostic factor of survival and tumor relapse. Yu et al[42] evaluated the association between cytokeratin 19 fragment (CYFRA 21-1) level and long-term OS, as well as the tumor clinicopathological features in NSCLC patients. It was clear that CYFRA 21-1 is a negative prognosis indicator and its high level of expression indicates higher TNM pathological stage (II + III + IV) in NSCLC. In advanced NSCLC, the level of CYFRA 21-1 seems to give more prognostic information than clinical TNM stage information.

Although the use of such markers in assessing the prognosis and monitoring the outcome of therapy in NSCLC is well known, the correlation between these and the sMICA levels is yet to be described. Since the classical markers dosed in NSCLC patients supply information about the evolution of the lung cancer, the hope is to add sMICA in the routine analysis in order to have another solid tool that can better clarify the development of disease. However, studies aimed to state a clear association between NSCLC markers and sMICA are yet to be performed.

# Significance of sMICA levels in patients with NSCLC

It has been demonstrated that the nonclassical MICA could activate NKG2D receptor expressed on the surface of NK cells and many cancer cells such as in lung cancer.[43–48] NK cells are activated by the binding of MICA and NKG2D, and so an NK cell could identify and lyse a target tumor cell through the expression of MICA on its surface. There

is also a relationship between expression level of MICA on tumor cells and antitumor efficacy. In patients with advanced NSCLC, high expression of MICA is a predictive factor for poor prognosis. In normal cells, MICA is usually not expressed, or is expressed in very low levels; however, these cells express MHC class I molecule. In normal cells, some events such as stress, viral infection, bacterial infection, and malignant transformation can lead to upregulation of MICA expression. Cells with overexpression of MICA are bound and eliminated by NKG2D-expressing cells like NK cells and CD8+ T lymphocytes, contributing to the immune surveillance. A recent study[49] demonstrated that MICA is also secreted in serum to generate a soluble form (sMICA). Patients with high levels of sMICA also have less MICA expressed on cellular membrane, and this process inhibits the antitumor effects of NK cells and CD8+ T cells by blocking their activation. The prognostic value of sMICA in NSCLC is not yet directly defined. There are evidences that in serum of patients with NSCLC, there are higher levels of sMICA compared to control group (Table 1) and sMICA levels are correlated with histological differentiation and tumor stage.

Wang et al[50] evaluated the prognostic significance of serum sMICA levels in NSCLC and the possibility to predict the presence of metastasis. In their study, 207 patients with NSCLC and 207 normal control individuals were enrolled. The NSCLC group patients had no previous history of other cancers. sMICA levels as calculated by ELISA test were higher in patients with NSCLC compared to their counterparts. The mean serum level was 143.52 ± 27.6 pg/mL in the NSCLC group and 32.4 ± 7.53 pg/mL in the control group ($p < 0.01$). Moreover, the data showed that patients with high serum sMICA level had a significantly lower 5-year OS rate (37.4.0% vs. 61.2%; $p = 0.012$) than those with low serum sMICA level, indicating that high sMICA levels are associated with poor prognosis in NSCLC.

Chen et al[51] investigated the clinical significance of MICA expression in patients with advanced NSCLC, aiming to clarify the correlation between MICA expression and the efficacy of cytokine-induced killer cell (CIK) therapy. A cohort of 222 patients with advanced NSCLC (defined as TNM stage III and IV) were enrolled in a time period of 5 years. Out of these 222 patients, 190 underwent treatments including chemotherapy, targeted therapy, radiotherapy, and CIK treatment. Of all the patients enrolled, 98.2% were positive for high MICA level. The median survival of patients with high expression of MICA was significantly shorter than those with a lower MICA expression ($p = 0.01$). Yet, among patients undergoing CIK therapy, those with high expression of MICA had a longer OS than patients with low expression (27 months vs. 13 months). In theory, the high MICA level activates NKG2D receptor on CIK cells and improves the effects of CIK therapy and thus the survival.

Jiang et al[52] investigated the sMICA/B level in different malignancies, such as gastrointestinal, lung, hematological, gynecological, and urological cancers. Out of the 495 patients, 70 had a lung cancer. sMICA and sMICB levels were assessed with ELISA test in the cancer group and in the healthy persons (control group). Tumoral patients had higher levels of sMICA and sMICB than control group. However, sMICA level was higher than sMICB, suggesting that sMICA would be more suitable as a marker for early diagnosis of lung cancer.

Holdenrieder et al[53] analyzed the sMICA level in malignant diseases, in order to state if it could be a useful marker in cancer. From the group of 512 patients with various malignancies, 19 had lung cancer, and 37 had benign lung disorders (tuberculosis, sarcoidosis, allergic, autoimmune, and infectious lung diseases, and others). A sandwich ELISA was used to measure the sMICA levels in individuals with cancer, healthy individuals, and subjects with a nonmalignant disease. Patients with lung cancer had a significantly higher value of sMICA than healthy individuals ($p < 0.0001$) and patients with benign lung disorders ($p < 0.0001$). Moreover, the sMICA level correlated significantly with metastasis

**Table 1** Studies comparing sMICA level in neoplastic versus control group

| Study | Sample and period | sMICA level in patients with NSCLC (pg/mL) | sMICA level in healthy controls (pg/mL) | *p* |
|---|---|---|---|---|
| Wang et al[50] | Venous blood of 207 patients with NSCLC and 207 normal control individuals, from February 2006 to October 2014 | 143.52 ± 276 | 32.4 ± 7.53 | <0.01 |
| Jiang et al[52] | Serum samples of 70 patients with NSCLC and 141 healthy controls, from October 2007 to December 2009 | 192.3 ± 258.2 | 44 ± 11.4 | 0.095 |
| Holdenrieder et al[53] | Serum samples of 19 patients with lung cancer and 62 healthy donors | 245 | 90 | <0.0001 |

**Abbreviations:** sMICA, soluble major histocompatibility complex class I polypeptide-related sequence A; NSCLC, non-small-cell lung cancer.

($p = 0.007$). Interestingly, in lung cancer patients, the sMICA level was also high in early stage.

Okita et al[54] analyzed the expression of MICA/B in response to cisplatin therapy in order to understand the regulatory mechanisms of MICA/B expression. Ninety-one patients with primary NSCLC undergoing anatomical surgical resection were enrolled in this study. Patients were excluded if they received a non-curative resection, had carcinoma in situ or an adenosquamous carcinoma, or had induction chemotherapy and/or radiotherapy. Of 91 patients, 28 had an overexpression of MICA/B, associated with a significant recurrence-free survival ($p = 0.037$) but with only a positive trend of OS ($p = 0.095$). Moreover, the in vitro data collected in this study showed that cisplatin enhances the immune response towards the tumor via upregulation of MICA/B.

He et al[55] also demonstrated that gefitinib influences the interaction between NK cells and lung cancer cells, via immune response. Using Cr release assay, CD107 assay, and interferon-γ secretion assay, they investigated the sensitivity of lung cancer cell lines A549 and H1975 to NK cells cytotoxicity in the presence of gefitinib. The results indicated that gefitinib increases the cytotoxic effects of NK cells towards the lung cancer, blocking the escape mechanisms mediated by the interaction between their NKG2D receptor and the MICA ligands on tumor cells.

Similarly, Park et al[56] found that sublethal dose of hemato-porphyrin-based photodynamic therapy (PDT) increased the expression of MICA/B in SW-900 lung cancer cells. This sublethal dose of hemato-porphyrin-based PDT also boosted the susceptibility of tumor cells towards NK cells cytotoxicity.

Okita et al[57] showed the different effects of gemcitabine and gefitinib as anticancer drugs in NSCLC and their regulation of NKG2DL. They demonstrated that cytotoxic drug-induced NKG2DLs may help in the clearance of tumor cells, but are potentially limited to the patients without NK cell dysfunction. In both cases, the OS of patients with NSCLC drugs was improved with a different mechanism: while gemcitabine treatment leads to an increased expression of MICA (NKG2DL) inducing phosphorylation of ATM, gefitinib causes a downregulation of expression, silencing EGFR using siRNA. In this study, the expression levels of MHC class I molecules and NKG2DL (MICA) were determined in five different NSCLC cell lines using flow cytometry. Materials included blood samples from buffy coats of blood, and cell culture was composed of human NSCLC cell lines: RERF-LC-AI, RERF-LC-KJ, and LC2/Ad, A549 cells, and PC-9 cell line. MICA was expressed in four of these five cell lines. It was reported that, in the A549 cell line, gemcitabine regulates the expression of MICA by DNA stress-induced ATM-ATR signaling; furthermore, the silencing of ATM gene could block gemcitabine-induced NKG2DL expression. Interestingly, EGFR-TKI gefitinib downregulated the expression of NKG2DL suggesting that NKG2DL expression also depends on other pathways, such as blocking the downstream signaling of EGFR, mainly PI3K-AKT pathway. In both cases, an important role was played by miR20a like how gefitinib decreases miR20a, which suppresses the expression of NKG2DLs.

Ren et al[58] illustrated in their study the relationship between estrogen and MICA/B expression in NSCLC. Human NSCLC cells (A549) and human lung adenocarcinoma cell line (LTEP-a2) were treated with high level of estradiol, noticing that MICA and MICB mRNA expression was upregulated after the exposure to the hormone. They also showed that estradiol promotes the secretory protein of MICA (sMICA) that, as we know, helps the tumor cells to evade the immune response mediated by NK cells.

Zhao et al[59] investigated the anticancer effects and mechanisms of immuno-chemotherapy of 5-fluorouracil (5-FU) and interleukin-2 (IL-2) on NSCLC through upregulation of NKG2DL and its promoter. Human NSCLC cells (A549) were used as cell culture for this study; they were transplanted subcutaneously into the right axilla of nude mouse and randomly divided into four groups: a control group was provided with normal saline, two groups with either 5-FU or IL-2, and the last one with a 5-FU and IL-2 combination. It was showed that the tumor volume, which was measured every three days, was particularly decreased in the 5-FU+IL-2 treatment group than in the others; moreover, the expression of MICA (presented as mean fluorescence intensity in each group) was 36.88 in 5-FU+IL-2 combination group and 12.9 and 17.12 in the 5-FU and IL-2 treatment group, respectively.

To support the effect of 5-FU+IL-2 immuno-chemotherapy on MICA, Zhao et al also investigated the promoter cloning and detection of promoter activity after incubation with 5-FU+IL-2. Results showed that MICA promoter activity was increased 1.45-, 1.79-, and 2.72-fold in the 5-FU, IL-2, and 5-FU+IL-2 group, respectively, compared with the control group. Yet, they also demonstrated that the number of NK cells in cultures was more upregulated in combination therapy group than in the monotherapy group; in fact, the number of NK cells in the spleen was 5.28% in the 5-FU+IL-2 treatment group, whereas that in the 5-FU- and IL-2-treated group was 3.67% and 4.89%, respectively.

## Future research directions

Although sMICA is a useful marker in surveillance of lung cancer, more studies are required to assess its different expression in various malignancies. MICA polymorphisms

are far to be fully understood, and currently, various studies are performed on population from different regions such as People's Republic of China and Iran; however, if the0polymorphism affects the expression of MICA on cells and tissues, and mRNA expression remains to be answered.[60–62]

Focusing on NSCLC therapy, the aim is to evaluate the possibilities of upregulating the expression of NKG2DLs on tumor cells, in order to increase their susceptibility to cytotoxic cells.

Overexpression of Rae1 and H60 (mouse NKG2DLs) stimulates the immune response against the tumor in vivo, and this effect mediated through NKG2D could be further boosted by administration of IL-21. However, due to the tumor immune escape mechanisms that weaken the MICA–NKG2D system, further research is necessary in order to fully learn the importance of these mechanisms in vivo and about how to overcome them before applying these gene therapy strategies to the treatment of NSCLC.

In future, the association between standard tumor markers and sMICA could also improve the diagnostic and prognostic yield of the single marker.

## Conclusion

High serum sMICA level in patients with NSCLC seems to be a poor prognostic factor being correlated with poor differentiation and advanced stage. However, the low specificity limits its role as a single prognostic marker of NSCLC, but its evaluation in addition to standard serum markers could improve the staging of NSCLC. Despite promising, the current studies are insufficient to assess the real efficiency of sMICA as a prognostic marker of NSCLC, and hence, further studies are required to validate it.

## Disclosure

The authors report no conflicts of interest in this work.

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